# Macroanalytical Procedures Manual (MPM)

# V-8. Spices, Condiments, Flavors, and Crude Drugs

# D. Supplemental Method for Saffron, Crocus sativus L.

### **April 2024**

Editor (s): Hans Loechelt-Yoshioka

Co-editor(s): Amy Barnes, Richard Haynos (Retired)

### **Contents**

D. Supplemental Method for Saffron, <i>Crocus sativus</i> L	2
(1) Scope	
(2) Applicable Documents	
(3) Defects	
(4) Procedure: Determination of Floral and Plant Waste (Extraneous Matter) and Foreign N	/latter 2
(5) Procedure: Determination of Insect-Damaged, Moldy, and Otherwise Reject Product	4
Figures	5
References Cited in Section	11
Additional Information	11
Revision History	11

#### D. Supplemental Method for Saffron, Crocus sativus L.

#### (1) Scope

This method supplements MPM Section V-8 A, Spices, Condiments, Flavors, and Crude Drugs, by describing procedures specific to saffron, *Crocus sativus* L. This supplement is applicable to saffron in the form of filaments and cut filaments (collectively referred here as saffron filaments) and not to saffron in powder form.

The definitions for saffron stigma, style, stamen, as well as extraneous matter and foreign matter (applicable to this supplemental method) can be found in the current edition of ISO 3632-1.

Saffron comes from the reddish stigma (or stigma with attached style) of the *C. sativus* L. flower (Figure V-8-D-1), which belongs in Iridaceae. Saffron is sometimes referred to as "red gold" since it is considered one of the most expensive spices (Cardone 2020).

#### (2) Applicable Documents

None

#### (3) Defects

As an agricultural crop, the *C. sativus* L plant is subject to the attack of field pests. Information on categories of defects that can potentially be found in these types of products are in MPM V-8 (A)(3).

Please note that in this supplemental method, the categories of extraneous matter and foreign matter are distinguished from each other. Refer to ISO 3632-1 and ISO 927 for definitions of these two categories. In this supplemental method, extraneous matter from *C. sativus* L. is referred to as "floral and plant waste." These definitions do not apply to other methods of the MPM, unless otherwise specified.

## (4) Procedure: Determination of Floral and Plant Waste (Extraneous Matter) and Foreign Matter

**a.** Sample Preparation – The representative sample consist of two (2) subsamples of 3-4 g each taken from the lot. Multiple consumer-ready retail containers could constitute a subsample. Take special care when removing the product from container(s) and avoid homogenizing because it may break the filaments. For multiple consumer-ready retail containers, carefully empty the contents from enough containers to attain the 3-4 g for analysis. For containers with larger quantities of product, carefully remove product from various locations throughout the container to attain the 3-4 g needed for analysis. Determine the initial weight of each subsample to the nearest 0.1 g to verify there is enough product for analysis and record results (see Table V-8-D-1 for recommended

reporting format). Examine total amount of product (3-4 g) from each of the two subsamples.

First, each subsample will need to be sorted into categories, then the sorted material will need to be examined. Next, the saffron filaments and extraneous matter will be further examined for insect damage, mold, and otherwise reject saffron. Foreign matter from animals or other (non-plant) sources will be identified and/or characterized.

**b.** *Visual Examination* –The product from each subsample must first be sorted. Then, the sorted material must be examined. For ease sorting and examination, the material should be spread out into a single layer on a glass Petri plate or watch glass. Glass should be used to minimize static charge issues.

<u>Sorting</u>: It is recommended to use magnification up to 10X for the initial sorting of the material. A 15 cm diameter glass Petri plate or watch glass usually accommodates the entire 3-4 g subsample. Place the glass Petri plate or watch glass either on a white or grey background. Using additional 15 cm diameter watch glasses or multiple glass Petri plates, divide the material of each subsample into the categories described below. Care should be taken while sorting the saffron, since it is typically very dry and easily broken.

- Saffron filaments (including saffron in cut filaments (Figure V-8-D-2) and saffron in filaments (Figure V-8-D-3)).
- Floral and plant waste (extraneous matter): This refers to the portions of the *C. sativus* L. flower without the reddish trumpet-shaped stigma (Figure V-8-D-4 A and B) and parts of the *C. sativus* L. plant other than the flower (Figure V-8-D-4 C). See definition in ISO 3632-1.
- Foreign matter from plants other than *C. sativus* L. (Figure V-8-D-5).
- Foreign matter: Any other matter from animal and non-animal origin. This
  may include insects, animal excreta, sand, plastic, etc. See definitions in
  ISO 3632-1 and ISO 927.

Place the sorted material in a single layer on the watch glasses or Petri plates. After the initial sorting, magnification higher than 10X may be used to confirm the categories listed above.

**c.** Classification of Sorted Material –Weigh material categorized as saffron filaments, floral and plant waste (extraneous matter), and foreign matter from plants other than *C. sativus* L. separately. Record the weights of each of these three categories (see Table V-8-D-1 for recommended reporting format).

Foreign matter from animal origin (e.g., dead insects, insect fragments, hairs, feather material, etc.) are further classified, counted, and identified to the lowest level possible. Other types of foreign matter (e.g., fibers, paint chips, plastic, etc.) are also further classified, counted, and described. Refer to MPM V-8 A (4)(c) and AOAC Official Method 970.66 Light and Heavy Filth for more information on classification these types of foreign matter.

**d.** *Report* – Use the recorded weight of material categorized as saffron filaments, floral and plant waste (extraneous matter), and foreign matter from plants other than *C. sativus* L., to determine percentages of each category based on the total weight of these combined categories. The total weight of these combined categories is calculated by adding the weights of the three categories together (see Table V-8-D-1 for an example of report).

Other foreign matter counted is reported by type with the appropriate quantitative figure. Tabulate results as outlined in Table V-8 A(4)d.

**Table V-8-D-1.** Recommended format for reporting categorized material.

	Subsample 1			Subsample 2		
	Initial weight* (g):			Initial weight* (g):		
Category	Saffron filaments	Floral/plant waste from C. sativus L. (Extraneous matter)	Foreign matter from plants other than C. sativus L.	Saffron filaments	Floral/plant waste from C. sativus L. (Extraneous matter)	Foreign matter from plants other than C. sativus L.
Weight (g)						
Total weight** (g)						
Percent (%)***						

<sup>\*</sup>Verify amount per subsample is between 3-4 g. Do not use this weight to calculate percentages.

## (5) Procedure: Determination of Insect-Damaged, Moldy, and Otherwise Reject Product

- **a. Sample Preparation** Examine material (saffron filaments, and floral and plant waste (extraneous matter)) categorized in (4)b. Transferring material to Petri plates with ruled filter paper can aid in the examination process.
- **b.** *Visual Examination* Using up to 10X magnification, examine the material on Petri plates for insect-damaged, moldy, or otherwise reject material. After this initial examination, higher magnification may be used to confirm the type of defect. Figures V-8-D-6 and V-8-D-7 show some examples of types of defects that can potentially be found on this product.
- **c.** Classification of Reject Material Classify and weigh rejected material. Refer to MPM V-8 5(c).
- **d.** *Report* Use the recorded weight of classified rejected material to determine their corresponding percentages. Calculate these percentages based on the total weight of

<sup>\*\*</sup>Total weight refers to the calculated sum (g) of categorized material (saffron filaments + floral/plant waste (extraneous matter) + foreign matter from plants other than C. sativus L.).

<sup>\*\*\*</sup>Percent of each category (based on total weight per subsample): [Weight of Category (g) / Total weight (g)] x 100

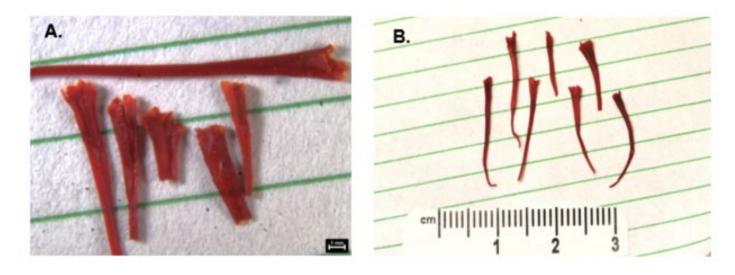
the two categories examined: saffron filaments + floral and plant waste (extraneous matter). Tabulate results (see section V-8 A for an example of reporting table).

#### **Figures**

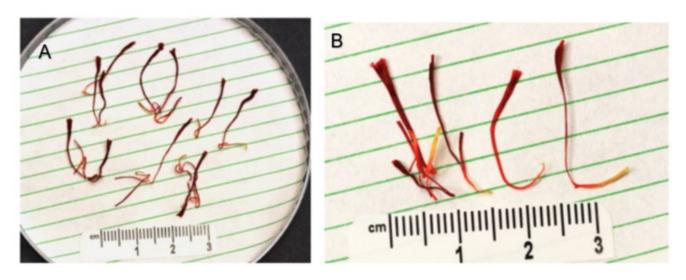
Figure V-8-D-1. Crocus sativus L. flower showing the reddish stigmas and styles. (Source: Photo	
courtesy of H. Loechelt-Yoshioka, FDA)	. 6
Figure V-8-D-2. A. and B. Saffron in cut filaments consisting of the reddish trumpet-shaped stigmas.	
Note: scale bar in A: 1mm. (Source: Photo's courtesy of H. Loechelt-Yoshioka, FDA)	. 6
Figure V-8-D-3. A and B are examples of saffron in filaments, showing the reddish trumpet-shaped	
stigma with attached style, which is usually yellowish white in color towards their base. (Source: Photo's	
courtesy of H. Loechelt-Yoshioka, FDA)	. 7
Figure V-8-D-4. Floral and plant waste (extraneous matter). A. and B. Floral waste from Crocus sativus	
L. Note that material shown do not have the attached reddish trumpet-shaped stigma, thus it represents	
floral waste. (Note: scale bar in <b>A</b> : 5 mm). <b>C</b> . Plant waste from <i>C. sativus</i> L. (scale bar: 1mm). (Source:	
Photo's courtesy of H. Loechelt-Yoshioka, FDA)	. 7
Figure V-8-D-5. Examples of foreign matter from plants other than <i>Crocus sativus</i> L. A. Dyed plant	
material mixed in with saffron filaments (scale bar: 1 mm). B. Close-up of the stigma of the foreign plant	
material (scale bar: 250 μm). <b>C.</b> Safflower, <i>Carthamus tinctorius</i> L., mixed in with saffron (scale bar: 1	
mm). <b>D.</b> Close-up of the safflower material (scale bar: 1 mm). (Source: Photo's courtesy of H. Loechelt-	
	. 8
Figure V-8-D-6. Examples of defects found in saffron. A. Insect feeding damage on saffron stigma	
(indicated by arrow; scale bar: 500 μm). <b>B.</b> Saffron filament with mold hyphae (indicated by arrow; scale	!
bar: 100 μm). <b>C.</b> Stigma with adhering white amorphous bird excreta (scale bar: 250 μm). <b>D.</b> Feather	
barbule (indicated by arrows) adhering to saffron stigma (scale bar: 75 μm). <b>E.</b> Saffron filament with	
encrusted dirt (50x). <b>F.</b> Saffron coated with an unidentified yellow substance (scale bar: 1.00 mm). <b>G.</b>	
Clumped saffron mixed with small stones (30x). (Source: Photo's courtesy of H. Loechelt-Yoshioka, FDA	-
	. 9
<b>Figure V-8-D-7.</b> Example of otherwise unfit saffron filament. <b>A.</b> Fake saffron (16x). <b>B.</b> Close-up of fake	
saffron (25x). <b>C.</b> Scanning electron microscope (SEM) view of the fake saffron. (Source: Photo's courtes	-
of H. Loechelt-Yoshioka, FDA).	10



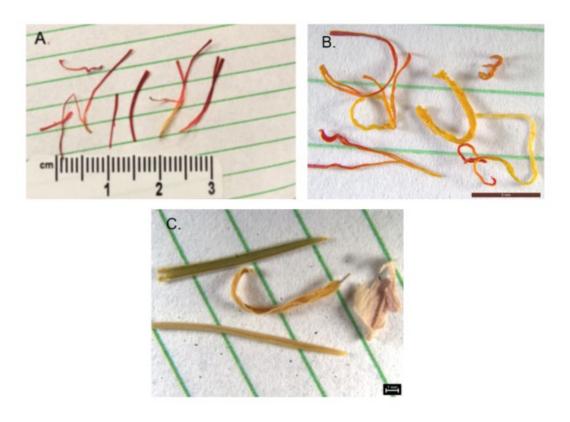
**Figure V-8-D-1.** *Crocus sativus* L. flower showing the reddish stigmas and styles. (Source: Photo courtesy of H. Loechelt-Yoshioka, FDA).



**Figure V-8-D-2. A.** and **B.** Saffron in cut filaments consisting of the reddish trumpet-shaped stigmas. Note: scale bar in **A**: 1 mm. (Source: Photo's courtesy of H. Loechelt-Yoshioka, FDA).

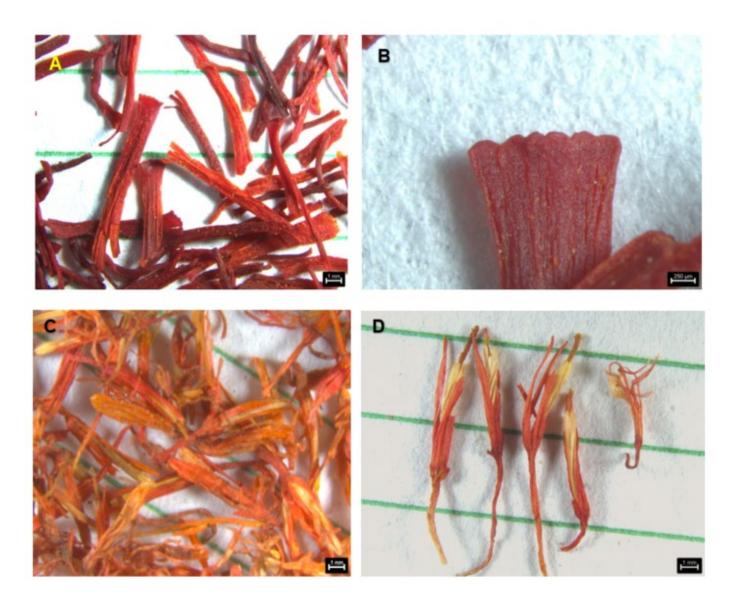


**Figure V-8-D-3. A** and **B** are examples of saffron in filaments showing the reddish trumpet-shaped stigma with attached style, which is usually yellowish white in color towards their base. (Source: Photo's courtesy of H. Loechelt-Yoshioka, FDA).



**Figure V-8-D-4.** Floral and plant waste (extraneous matter). **A.** and **B.** Floral waste from *Crocus sativus* L. Note that material shown do not have the attached reddish trumpet-shaped stigma, thus it represents floral waste. (Note: scale bar in **A**: 5 mm). **C**. Plant waste from *C. sativus* L. (scale bar: 1 mm). (Source: Photo's courtesy of H. Loechelt-Yoshioka, FDA).

MPM: V-8-D



**Figure V-8-D-5.** Examples of foreign matter from plants other than *Crocus sativus* L. **A.** Dyed plant material mixed in with saffron filaments (scale bar: 1 mm). **B.** Close-up of the stigma of the foreign plant material (scale bar: 250 μm). **C.** Safflower, *Carthamus tinctorius* L., mixed in with saffron (scale bar: 1 mm). **D.** Close-up of the safflower material (scale bar: 1 mm). (Source: Photo's courtesy of H. Loechelt-Yoshioka, FDA).

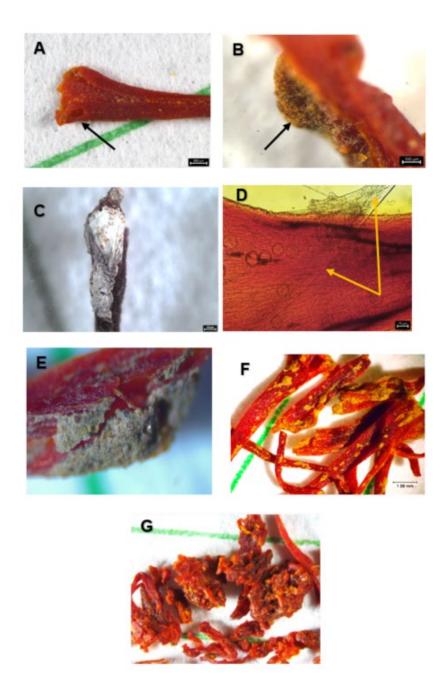
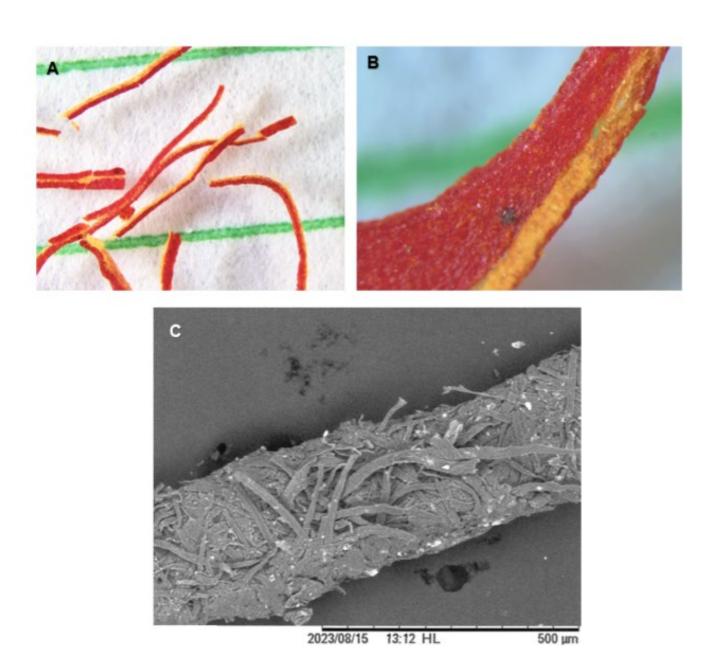


Figure V-8-D-6. Examples of defects found in saffron. A. Insect feeding damage on saffron stigma (indicated by arrow; scale bar: 500 μm). B. Saffron filament with mold hyphae (indicated by arrow; scale bar: 100 μm). C. Stigma with adhering white amorphous bird excreta (scale bar: 250 μm). D. Feather barbule (indicated by arrows) adhering to saffron stigma (scale bar: 75 μm). E. Saffron filament with encrusted dirt (50X). F. Saffron coated with an unidentified yellow substance (scale bar: 1.00 mm). G. Clumped saffron mixed with small stones (30X). (Source: Photo's courtesy of H. Loechelt-Yoshioka, FDA)



**Figure V-8-D-7.** Example of otherwise unfit saffron filament. **A.** Fake saffron (16X). **B.** Close-up of fake saffron (25X). **C.** Scanning electron microscope (SEM) view of the fake saffron. (Source: Photo's courtesy of H. Loechelt-Yoshioka, FDA).

### **References Cited in Section**

Cardone, L., Castronuovo, D., Perniola, M., Cicco, N., Candido, V. (2020). *Saffron (Crocus sativus L.), the King of Spices: An Overview.* Scientia Horticulturae 272:1-13. <a href="https://www.sciencedirect.com/science/article/abs/pii/S0304423820303885">https://www.sciencedirect.com/science/article/abs/pii/S0304423820303885</a>

ISO (2010) International Standard: Spices-Saffron (*Crocus sativus* L.) – part 2: Test Methods. ISO 3632-2:2010(E).

ISO (2011) International Standard: Spices-Saffron (*Crocus sativus* L.) – part 1: Specification. ISO 3632-1:2011(E).

ISO (2009) International Standard: Spices and Condiments – Determination of extraneous matter and foreign matter content. ISO 927:2009 (E).

AOAC Official Method 970.66B(i) Light and Heavy Filth General. *Official Methods of Analysis of AOAC INTERNATIONAL*, Chapter 16.

## **Additional Information**

Informational articles not cited in the above section, but still useful:

Lowell, G. (1964). Saffron Adulteration. *Journal of Association of Official Agricultural Chemists*. 47(3): 538-538.

U.S. FDA (1964) *Saffron Adulteration by Gertrude Lowell.* Laboratory Information Bulletin (LIB), 16.

## **Revision History**

Version No.	Purpose of change	Date
V0	New process	2024